DRAFT: Nutrient Holding Time Study Report

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Introduction

The Surface Water Ambient Monitoring Program (SWAMP) collects samples for nutrient analysis to help characterize the overall health of a water body. Current holding times required by SWAMP for sample analysis for soluble reactive phosphorus (SRP), nitrite (NO_2^-), nitrate + nitrite ($NO_3^- + NO_2^-$), ammonia (NH_4^+), nitrate (NO_3^-), total nitrogen (TN), and total phosphorus (TP) are 48 hours. Transferring samples from the water body to the laboratory for analysis within the required holding time is not always feasible because of logistical and budget concerns. Some sampling locations are so remote that samplers are required to hike in, take the sample, hike out, and then either take the sample the sample to a nearby lab or ship the sample to the lab via overnight delivery. Another problem is that even if the water body is accessible there may not be a lab close enough to the water body to perform the analyses within the required holding times requiring the samples to be shipped overnight to the lab. Acid preservation is useful for extending the holding times of samples with high concentrations of nutrients, but acid preservation may compromise the integrity of samples containing low levels of nutrients.

Phosphorus and Nitrogen naturally exist in several forms within aquatic ecosystems as food sources for animal and plant growth and are more commonly referred to as nutrients. Nutrients naturally exist in surface waters as a food source necessary for plant and animal growth (Mueller 1996). When nutrients are overabundant in an aquatic ecosystem eutrophication occurs which causes algal blooms that lead to unpleasant odors and tastes, decreased dissolved oxygen levels, and fish kills (Mueller 1996).

The purpose of this study is to evaluate the holding times for samples for nutrient analysis. Data from this study will be used to determine whether the required holding times currently recommended by the US EPA can be extended without compromising the integrity of the samples.

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Experimental Design

Soluble Reactive Phosphorus, Nitrite, Ammonia, and Nitrate + Nitrite

Upon collection, samples being analyzed for SRP, NO_2^-, NH_4^+, and $NO_3^- + NO_2^-$ were filtered. Two forms of preservation were used: refrigeration (at 4℃) and freezing (at -20℃); samples for refrigeration were placed on wet ice in the field and samples for freezing were placed on fry ice in the field. Samples were analyzed at 48 hour, 4 day, 7 day, and 28 day hold times with the 48 hour hold time being the reference point for the proposed extended hold times to be compared to. The stannous chloride method (SM4500-P D) was used to spectroscopically determine SRP. The vanadium chloride (VCl_3) method (Doane 2003) was used to spectroscopically determine $NO_3^- + NO_2^-$. EPA method 353.2 was used to determine NO_2^-. The Berthelot reaction was used to spectroscopically determine NH_4^+. Laboratory quality assurance/quality control followed the SWAMP protocols set by the California State Water Resources Control Board.

Total Nitrogen and Total Phosphorus

All samples for total nitrogen (TN) and total phosphorus (TP) were refrigerated and evaluated at 48 hour, 4 day, 7day, and 28 day holding times. Samples for TN or TP were not filtered. Oxidation of both TN and TP was done using 1% potassium persulfate solution (SM4500N-C) before determination of analyte amounts. The vanadium chloride (VCl_3) method (Doane 2003) was used to spectroscopically determine TN. The stannous chloride method (SM4500-P D) was used to spectroscopically determine TP.

Statistical Analysis

The mean values of results measured at 4 days, 7 days, and 28 days were compared with the mean value of results measured within 48 hours of sample collection (reference result) to determine stability of each analyte over time. An Analyte was considered stable if the percent recovery between the mean value analyzed at a given time (either 4 days, 7 days, or 28 days in this study) and the mean reference value, taken at less than 48 hours, was less than 10%.

Sampling Locations

Two locations in three different regions were chosen for a total of six sampling sites. Samples in the Lahontan Region were taken from West Fork of the Carson River below Willow Creek (WFCR) and Upper Truckee River at South Upper Truckee Road (UTR). Samples in the Lahontan Region were spiked with

0.025mg/L of SRP, NO_2^-, NH_4^+, and NO_3^- because of low nutrient levels historically exhibited by those water bodies. Samples in the Central Valley Region were taken from the San Joaquin River (SJR) at Vernalis and the Sacramento River (SRF) at Freeport; these water bodies have historically exhibited low- to mid-level nutrient concentrations. Samples from the Central Coast Region were taken from Orcutt Creek (OC) in San Luis Obispo and Franklin Creek (FC) in Carpenteria; these water bodies have historically exhibited high-level nutrient concentrations. Table 1 shows the historic ranges of constituent concentrations for the sampling sites of the study.

Results

Soluble Reactive Phosphate

Soluble Reactive Phosphate (SRP), also known as orthophosphate, was analyzed using Standard Method 4500P-D. Samples that were preserved with freezing can have their holding times extended up to 4 days. Samples that were preserved through refrigeration can have their holding times extended up to 4 days. Table 2 shows the stability of SRP at different ranges at the different holding times. Samples with an SRP concentration greater than 0.050mg/L (the reporting limit) showed analyte stability up to seven days after sampling. Table 3 shows the soluble reactive phosphorus data for both freezing and refrigeration preservations. With the current data there is no information on samples with SRP concentrations between 0.060mg/L and 0.400mg/L and there is no data for samples with more than 0.500mg/L. Further studies need to be done to analyze analyte stability over time with a greater variety of SRP concentrations.

Figure 1 shows the percent recovery between the mean reference value (48 hour holding time) and the mean results taken at 4 day, 7 day, and 28 day holding times from samples preserved by freezing. Mean results from all six locations had percent recoveries of less than 10% for samples analyzed at 4 days. At 7 days percent recoveries for five locations were less than 10% with Franklin Creek having a percent recovery that was greater than 10%. The mean result for Franklin Creek at seven days was 0.019 mg/L and the resulting percent recovery was 11.8%.

Figure 2 shows the percent recovery between the mean reference value (48 hour holding time) and the mean results taken at 4 day, 7 day, and 28 day holding times from samples preserved by refrigeration. Mean results from all six sampling locations had percent recoveries that were less than 10% at 4 days. At 7 days only three of the mean results had percent recoveries that were less than 10%: West Fork Carson River, San Joaquin River, and Orcutt Creek. Mean results whose percent recoveries were greater than 10% at 7 days ranged from 0.020 to 0.034 mg/L while samples with percent recoveries that were less than 10% ranged from 0.023 to 0.465 mg/L.

Nitrite

Nitrite (NO_2^-) samples were analyzed using EPA Method 353.2. Samples taken from the Sacramento River at Freeport exhibited nitrite concentrations below the minimum detection limit (0.010 mg/L) therefore no conclusions were drawn from that data. Table 4 shows the stability of nitrite in samples at different ranges over time. Nitrite samples may not have their holding times extended past the 48 hour holding for either preservation method. In the nitrite levels tested from samples that were preserved by freezing nitrite showed stability for four days at three of the analyte ranges measured: 0.019mg/L - 0.029mg/L, 0.138mg/L - 0.144mg/L, 0.239mg/L - 0.248mg/L. Samples with nitrite concentrations in the 0.028mg/L - 0.036mg/L range, however, did not show analyte stability after 48 hours. In samples that were preserved with refrigeration nitrite only showed stability after the 48 hour hold time in samples with nitrite concentrations in the following ranges: 0.138mg/L - 0.144mg/L and 0.239mg/L - 0.248mg/L; samples with smaller nitrite concentrations did not show analyte stability over time. Further studies need to be done to evaluate the stability of nitrite over a greater range of concentrations. Table 5 shows the nitrite data for both freezing and refrigeration preservations.

Figure 3 shows the percent recovery between the mean reference value (48 hour holding time) and the mean results taken at 4 day, 7 day, and 28 day holding times from samples preserved by freezing. Mean results from four of the sampling locations had percent recoveries that were less than 10% at 4 days. At San Joaquin River the mean value of 0.032 mg/L resulted in a percent recovery of -11.1%. At 7 days three of the mean results had percent recoveries that were greater than 10% and those results were 0.023 mg/L (-20.7%), 0.019 mg/L (-26.9%), and 0.028 mg/L (-22.2%) all near or below the reporting limit value. At seven days the values within the 10% range were 0.141 mg/L and 0.243 mg/L. At 28 days the results that produced percent recoveries that were greater than 10% ranged from 0.027 mg/L to 0.248 mg/L but only the values that were greater than five times the reporting limit consistently had percent recoveries that were greater than 10% over the 28 day period.

Figure 4 shows the percent recovery between the mean reference value (48 hour holding time) and the mean results taken at 4 day, 7 day, and 28 day holding times from samples preserved by refrigeration. Mean results from two of the sampling sites had percent recoveries that were not within 10% of the reference result at 4 days. At 7 days three of the samples had percent recoveries that were not within 10% of the reference result. Only the samples from Orcutt Creek and Franklin Creek consistently had percent recoveries that were less than 10% over the 28 day period and the nitrite concentrations from those sampling sites ranged between 0.138 mg/L and 0.246 mg/L, well above the reporting limit.

Nitrate + Nitrite

Nitrate + Nitrite (NO_3^- + NO_2^-) was evaluated using the vanadium chloride method which was originally developed for blood serum analysis (Miranda 2001) and later applied to other sample types. The vanadium chloride method was shown to be comparable to EPA method 353.2 which uses granulated copper-cadmium instead of vanadium chloride for the reduction of nitrate to nitrite in a study done by Timothy Doane and William Horwàth (Doane 2003). Samples that were preserved by freezing may be held up to 4 days without compromising the nitrate + nitrite levels at the concentrations tested. Samples that were preserved by freezing can have their holding times extended up to 28 days when analyte concentrations are above the reporting limit of 0.050 mg/L. Table 6 shows the stability of nitrate + nitrite at different concentrations at each holding time. This study covered a limited range of concentrations and further studies need to be done to determine the stability of nitrate + nitrite at concentrations between 0.5 mg/L and 24 mg/L. Samples that were preserved by refrigeration cannot have their holding times extended. Nitrate + nitrite concentrations above the reporting limit show stability through 28 days. Table 7 shows the nitrate + nitrite data for both freezing and refrigeration preservations.

Figure 5 shows the percent recovery between the mean reference value (48 hour holding time) and the mean results taken at 4 day, 7 day, and 28 holding times from samples preserved by freezing. Mean results had percent recoveries within 10% of the reference result at 4 days at all six sampling stations. At 7 days mean results for five locations had percent recoveries that were less than 10% with Sacramento River producing a mean value with a percent recovery greater than 10%. The mean result for Sacramento River at 7 days was 0.022 mg/L and the resulting percent recovery was 15.8%. At 28 days the only mean result leading to a percent recovery that was greater than 10% was from Sacramento River again at 0.024 mg/L for a percent recovery of 26.3%.

Figure 6 shows the percent recovery between the mean reference value (48 hour holding time) and the mean results taken at 4 day, 7 day, and 28 holding times from samples preserved by refrigeration. Mean results resulted in percent recoveries that were less than 10% at five of the six sampling sites at 4 days. The mean result at Sacramento River was 0.020 mg/L at 4 days with a percent recovery of 17.6%; all other samples had nitrate + nitrite concentrations greater than or equal to 0.045mg/L. The percent recovery at 7 days and 28 days of samples collected at Sacramento River were 29.4% and 35.3%, respectively. The percent recoveries of Upper Truckee River and West Fork of Carson River were 13.9% and 12.2%, respectively, at 28 days. The mean result at Upper Truckee River was 0.49 mg/L and the mean result at West Fork Carson River was 0.046 mg/L.

Ammonia

Ammonia (NH_4^+) was evaluated using the Berthelot reaction using Sodium Salicylate instead of phenol. A study done by Verdouw shows that using Sodium Salicylate in place of phenol is comparable to the Nessler method (EPA method 350.2) (Verdouw 1977). The holding times for ammonia samples should not be extended regardless of which preservation method was used. Data shows samples with ammonia concentrations near the reporting limit of 0.020mg/L resulted in percent recoveries of greater than 10%. Samples from Orcutt Creek had ammonia concentrations around 0.100mg/L and these samples showed analyte stability up to 28 days when frozen and up to 7 days when refrigerated. Table 8 shows the stability of ammonia at different concentrations at each holding time. Table 9 shows the ammonia data for both freezing and refrigeration preservations. Ammonia concentrations from five of the six sites range from 0.018 mg/L to 0.026 mg/L while ammonia concentrations at the sixth site were greater than 0.100 mg/L. Further studies need to be done to determine analyte stability at a greater range of ammonia concentrations.

Figure 7 shows the percent recovery between the mean reference value (48 hour holding time) and the mean results taken at 4 day, 7 day, and 28 holding times from samples preserved by freezing. As can be seen only one site had samples that consistently had percent recoveries that were less than 10%. Ammonia concentrations from all six sites ranged from 0.014 mg/L to 0.119 mg/L; the site with samples with ammonia concentrations greater than 1mg/L was the site with percent recoveries consistently less than 10%.

Figure 8 shows the percent recovery between the mean reference value (48 hour holding time) and the mean results taken at 4 day, 7 day, and 28 holding times from samples preserved by refrigeration. As can be seen no site had samples that consistently had percent recoveries that were less than 10%. Ammonia concentrations from all six sites ranged from 0.006 mg/L to 0.119 mg/L.

Nitrate

Nitrate (NO_3^-) was calculated by subtracting the mean results of the Nitrite analysis from the mean results of Nitrate + Nitrite analysis. Samples that are preserved with either freezing or refrigeration preservation methods cannot have their holding times extended. Samples with nitrate concentrations greater than 1 mg/L were able to show analyte stability up to 28 days. Table 10 shows nitrate stability at different concentrations at each holding time. Further studies need to be done to determine analyte stability at concentrations between 0.025 mg/L and 1.5 mg/L. Table 11 shows the nitrate data for both freezing and refrigeration preservations.

Figure 9 shows the percent recovery between the mean reference value (48 hour holding time) and the mean results taken at 4 day, 7 day, and 28 day holding

times from samples preserved by freezing. The mean results for samples from the San Joaquin River, Franklin Creek, and Orcutt Creek consistently had percent recoveries that were less than 10%. The mean values at these sites ranged from 1.438 mg/L to 32.401 mg/L while the mean values for sites with percent recoveries that were greater than 10% ranged from 0.012 mg/L to 0.024 mg/L.

Figure 10 shows the percent recovery between the mean reference value (48 hour holding time) and the mean results taken at 4 day, 7 day, and 28 day holding times from samples preserved by refrigeration. The San Joaquin River, Franklin Creek, and Orcutt Creek consistently had percent recoveries that were less than 10%. The mean values at these sites ranged from 1.452 mg/L to 32.385 mg/L while the mean values for site with percent recoveries that were greater than 10% ranged from 0.010 mg/L to 0.023 mg/L.

Total Nitrogen

Total Nitrogen was determined as nitrate using the vanadium chloride method. Refrigeration was the only preservation method used for Total Nitrogen and the samples were not filtered. Total Nitrogen samples can have their holding times extended up to 28 days. The outliers that are shown in Figure 11 at -11% at 4 days and 25% at 28 days can be thrown out because of the high bias of 116% recovery and a high RSD of 24%. Figure 11 shows the percent recovery between the mean reference value (48 hour holding time) and the mean results taken at 4 day, 7 day, and 28 day holding times from and only two samples have percent recoveries that were less than 10%. Table 12 shows total nitrogen stability at different concentrations at each holding time. Table 13 shows the total nitrogen data preserved by refrigeration.

Total Kjeldahl Nitrogen (TKN) can be calculated by subtracting the result of Nitrate + Nitrite from the result of Total Nitrogen. The holding times for TKN values that are calculated are reliant upon the holding times for the analytes that they are calculated from, therefore the holding times for TKN results that are calculated from Nitrate + Nitrite values can only be extended to up to 4 days. The holding time for TKN that is measured using the Kjeldahl digestion is 7 days.

Total Phosphorus

Total Phosphorus was determined using SM4500-P D and preserved by refrigeration. The holding time for Total Phosphorus cannot be extended past the US EPA recommended holding time. Only three sites had mean values that resulted in percent recoveries that were less than 10% at the 4 day holding time. At 7 days only two sites had mean values that resulted in percent recoveries that were less than 10%. At 28 days only one site had a percent recovery that was less than 10%. Figure 12 shows the percent recovery between the mean reference value (48 hour holding time) and the mean results taken at 4 day, 7

day, and 28 day holding times. Table 14 shows total phosphorus stability at different concentrations at each holding time. Table 15 shows the total phosphorus data preserved by refrigerating.

Conclusions

This study has found that the holding times of some constituents that are regularly sampled by SWAMP programs can be extended past the SWAMP required holding times. The holding time for SRP may be extended up to 4 days for samples that are either refrigerated or frozen. As can be seen in Table 2 further studies need to be conducted to evaluate the stability of SRP at a wider range of concentrations. The holding time for nitrite cannot be extended with either method of preservation. Holding times for Nitrate + Nitrite may be extended up to 4 days when samples are frozen. Samples that were refrigerated cannot have their holding times extended for nitrate + nitrite. Holding times for Ammonia cannot be extended from the <48 hour holding time that is currently recommended by the US EPA for either preservation method. Nitrate holding time cannot be extended regardless of which preservation method is used. Total Nitrogen holding time may be extended up to 28 days regardless of which preservation method is used. Total Phosphorus holding time cannot be extended past the <48 hour hold time that is currently recommended by US EPA regardless of which preservation method is used. Table 9 summarizes the findings of the nutrient holding time study.

Next Steps

It is recommended that analyses are done using more water samples to provide data on a wider range of concentrations for each analyte. Samples with concentrations of SRP between 0.060 mg/L and 0.400 mg/L as well as concentrations greater than 0.500 mg/L should be analyzed at longer hold times to determine analyte stability. Samples with concentrations of Nitrite between 0.025 mg/L and 0.130 mg/L as well as samples greater than 0.250 mg/L should be analyzed at longer hold times to determine analyte stability. Samples with concentrations of Nitrate + Nitrite between 0.050 mg/L and 24 mg/L should be analyzed at longer hold times to verify analyte stability at more analyte concentrations. Samples that have concentrations of Ammonia greater than 0.030 mg/L should be analyzed at longer hold times to verify analyte stability. Samples containing Nitrate at concentrations less than 1 mg/L and at concentrations between 1.5 mg/L and 23 mg/L should be evaluated at each hold time to show analyte stability at a greater range of concentrations. More studies could also be done to evaluate the holding times of total phosphorus at a wider range of concentrations.

Low-level nutrient samples should be spiked at specific levels within the calibration curve at concentrations of expected optimal instrumentation performance. The first set of samples should have a concentration corresponding to the 150 µg/L level. That provides strong signal to noise ratios

for assessing holding times at higher concentration samples while staying within the typical range of the calibration curve. The second set of samples should be spiked at 50 µg/L, corresponding to the level of continuing calibration verification (CCV) samples.

Citations

Correll, D. L., 1999. Phosphorous: A Rate Limiting Nutrient in Surface Waters. Poultry Science 78:674-682

Doane, T.A.; Horwath, W.R. 2003. Spectrophotometric Determination of Nitrate with a Single Reagent. *Anal. Lett*. *36*, 2713-2722.

Mueller, D.K., and Helsel, D.R., 1996, Nutrients in the Nation's Waters--Too Much of a Good Thing?: U.S. Geological Survey Circular 1136, on line at <URL: http://pubs.usgs.gov/circ/circ1136/>, updated September 08, 2008.

Verdouw, H. C. J. A. van Echteld and E. M. J. Dekkers. 1977. Ammonia Determination Based on Indophenol Formation with Sodium Salicylate. *Water Research* Vol. 12, pp. 399 to 402.

Table 1. Summary of Sampling Sites and Historic Ranges in Constituent Concentrations for 2008 SRP/Nitrite Holding Time Study

	SRP (mg/L)	Nitrite (mg/L)	Nitrate + Nitrate (mg/L)	Ammonia (mg/L)	Nitrate (mg/L)	Total N (calculated value) (mg/L)	TP (mg/L)
Tahoe Region*							
West Carson River below Willow Creek	NA	< .008	0.001 to 0.084	NA	NA	NA	0.012 to 0.061
Upper Truckee River at South Upper Truckee Road	0.004 to 0.023	NA	0.006 to 0.046	ND to 0.011	NA	NA	0.011 to 0.047
Central Valley Region							
Sacramento River Freeport/Hood	about 0.02	NA	0.04-0.50 as mg/L	0.05 to 0.19	ND to 12.4	NA	0.31
San Joaquin River at Vernalis	0.06 to 0.48	0.01 to 0.04	0.04-3.0 mg/L	ND to 0.5	ND to 2.22	0.46 to 3	0.1 to 12.12
Central Coast Region							
Orcutt Creek	0.0198 to 0.84	ND to 1.1	NA	ND to 2.8	24 to 35 mg/L	19.6 to 43	NA
Franklin Creek in Carpinteria	ND to 1.9	ND to 0.33	NA	ND to 0.3116	22 to 24 mg/L	2.1 to 28	NA

* Tahoe sites were spiked 25 ug/L SRP, NO3, NO2, NH4 (providing 50-ug/L NO3+NO2 and 75-ug/L TN)

Table 2. SRP Stability over time at Specific Ranges	Frozen Preservation			Refrigerated Preservation		
Range of SRP Concentration	4 Days	7 Days	28 Days	4 Days	7 Days	28 Days
Below Reporting Limit (0.050mg/L)	Yes	No	No	Yes	No	No
0.050mg/L - 0.060mg/L	Yes	Yes	Yes	Yes	Yes	No
0.400mg/L - 0.500mg/L	Yes	Yes	Yes	Yes	Yes	Yes

Table 3. Soluble Reactive Phosphorus

		UTR		WFCR		SJR		SRF		FC		OC	
	Time	SRP (mg/L)	Percent Recovery (%)	SRP (mg/L)	Percent Recovery (%)	SRP (mg/L)	Percent Recovery (%)	SRP (mg/L)	Percent Recovery (%)	SRP (mg/L)	Percent Recovery (%)	SRP (mg/L)	Percent Recovery (%)
Field Filtered and Frozen	<48 hours	0.033		0.023		0.059		0.028		0.017		0.446	
	4 days	0.035	6.06	0.025	8.70	0.064	8.47	0.029	3.57	0.018	5.88	0.465	4.26
	7 days	0.036	9.09	0.024	4.35	0.058	-1.69	0.030	7.14	0.019	11.76	0.473	6.05
	28 days	0.035	6.06	0.028	21.74	0.059	0.00	0.027	-3.57	0.018	5.88	0.438	-1.79
Field Filtered and Refrigerated	<48 hours	0.029		0.025		0.062		0.027		0.018		0.443	
	4 days	0.030	3.45	0.023	-8.00	0.061	-1.61	0.027	0.00	0.019	5.56	0.473	6.77
	7 days	0.034	17.24	0.023	-8.00	0.060	-3.23	0.032	18.52	0.020	11.11	0.465	4.97
	28 days	0.031	6.90	0.022	-12.00	0.054	-12.90	0.026	-3.70	0.014	-22.22	0.415	-6.32

Table 4. Nitrite Stability over time at Specific Ranges	Frozen Preservation			Refrigerated Preservation		
Range of Nitrite Concentration	4 Days	7 Days	28 Days	4 Days	7 Days	28 Days
0.019mg/L - 0.029mg/L	Yes	No	No	No	No	No
0.028mg/L - 0.036mg/L	No	No	No	No	No	No
0.138mg/L - 0.144mg/L	Yes	Yes	Yes	Yes	Yes	Yes
0.239mg/L - 0.248mg/L	Yes	Yes	Yes	Yes	Yes	Yes

Table 5. Nitrite = not usable data because values are below the minimum detection limit

		UTR		WFCR		SJR		SRF		FC		OC	
	Time	Nitrite (mg/L)	Percent Recovery (%)	Nitrite (mg/L)	Percent Recovery (%)	Nitrite (mg/L)	Percent Recovery (%)	Nitrite (mg/L)	Percent Recovery (%)	Nitrite (mg/L)	Percent Recovery (%)	Nitrite (mg/L)	Percent Recovery (%)
Field Filtered and Frozen	<48 hours	0.029		0.026		0.036		0.007		0.140		0.243	
	4 days	0.028	-3.45	0.025	-3.85	0.032	-11.11	0.002	-71.43	0.139	-0.71	0.240	-1.23
	7 days	0.023	-20.69	0.019	-26.92	0.028	-22.22	-0.002	-128.57	0.141	0.71	0.243	0.00
	28 days	0.028	-3.45	0.027	3.85	0.032	-11.11	0.003	-57.14	0.142	1.43	0.248	2.06
Field Filtered and Refrigerated	<48 hours	0.029		0.026		0.036		0.007		0.140		0.241	
	4 days	0.025	-13.79	0.025	-3.85	0.032	-11.11	0.004	-42.86	0.138	-1.43	0.239	-0.83
	7 days	0.022	-24.14	0.021	-19.23	0.028	-22.22	-0.001	-114.29	0.140	0.00	0.241	0.00
	28 days	0.027	-6.90	0.027	3.85	0.033	-8.33	0.003	-57.14	0.144	2.86	0.246	2.07

Table 6. Nitrate + Nitrite Stability over time at Specific Ranges	Frozen Preservation			Refrigerated Preservation		
Range of Nitrate + Nitrite Concentration	4 Days	7 Days	28 Days	4 Days	7 Days	28 Days
0.017mg/L - 0.024mg/L	Yes	No	No	No	No	No
0.041mg/L - 0.046mg/L	Yes	Yes	Yes	Yes	Yes	No
1.474mg/L - 1.506mg/L	Yes	Yes	Yes	Yes	Yes	Yes
24mg/L - 26mg/L	Yes	Yes	Yes	Yes	Yes	Yes
30mg/L - 33mg/L	Yes	Yes	Yes	Yes	Yes	Yes

Table 7. Nitrate + Nitrite

		UTR		WFCR		SJR		SRF		FC		OC	
	Time	Nitrate + Nitrite (mg/L)	Percent Recovery (%)	Nitrate + Nitrite (mg/L)	Percent Recovery (%)	Nitrate + Nitrite (mg/L)	Percent Recovery (%)	Nitrate + Nitrite (mg/L)	Percent Recovery (%)	Nitrate + Nitrite (mg/L)	Percent Recovery (%)	Nitrate + Nitrite (mg/L)	Percent Recovery (%)
Field Filtered and Frozen	<48 hours	0.048		0.042		1.474		0.019		25.786		32.471	
	4 days	0.049	2.08	0.043	2.38	1.484	0.68	0.020	5.26	25.686	-0.39	32.571	0.31
	7 days	0.046	-4.17	0.042	0.00	1.493	1.29	0.022	15.79	25.157	-2.44	32.314	-0.48
	28 days	0.048	0.00	0.046	9.52	1.500	1.76	0.024	26.32	24.029	-6.81	30.486	-6.11
Field Filtered and Refrigerated	<48 hours	0.043		0.041		1.494		0.017		25.400		32.400	
	4 days	0.045	4.65	0.045	9.76	1.484	-0.67	0.020	17.65	25.429	0.11	32.000	-1.23
	7 days	0.045	4.65	0.041	0.00	1.496	0.13	0.022	29.41	25.271	-0.51	32.557	0.48
	28 days	0.049	13.95	0.046	12.20	1.506	0.80	0.023	35.29	24.471	-3.66	30.771	-5.03

Table 8. Ammonia Stability over time at Specific Ranges	Frozen Preservation			Refrigerated Preservation		
Range of Ammonia Concentration	4 Days	7 Days	28 Days	4 Days	7 Days	28 Days
0.014mg/L - 0.021mg/L	No	No	No	Yes	No	No
0.023mg/L - 0.032mg/L	No	No	No	No	No	No
0.099mg/L - 0.119mg/L	Yes	Yes	Yes	Yes	Yes	No

Table 9. Ammonia

		UTR		WFCR		SJR		SRF		FC		OC	
	Time	Ammonia (mg/L)	Percent Recovery (%)	Ammonia (mg/L)	Percent Recovery (%)	Ammonia (mg/L)	Percent Recovery (%)	Ammonia (mg/L)	Percent Recovery (%)	Ammonia (mg/L)	Percent Recovery (%)	Ammonia (mg/L)	Percent Recovery (%)
Field Filtered and Frozen	<48 hours	0.026		0.026		0.021		0.019		0.026		0.114	
	4 days	0.029	11.54	0.024	-7.69	0.016	-23.81	0.021	10.53	0.026	0.00	0.115	0.88
	7 days	0.023	-11.54	0.023	-11.54	0.014	-33.33	0.014	-26.32	0.017	-34.62	0.119	4.39
	28 days	0.031	19.23	0.032	23.08	0.017	-19.05	0.018	-5.26	0.021	-19.23	0.112	-1.75
Field Filtered and Refrigerated	<48 hours	0.024		0.026		0.020		0.018		0.022		0.114	
	4 days	0.028	16.67	0.025	-3.85	0.019	-5.00	0.019	5.56	0.021	-4.55	0.119	4.39
	7 days	0.021	-12.50	0.023	-11.54	0.014	-30.00	0.015	-16.67	0.020	-9.09	0.117	2.63
	28 days	0.030	25.00	0.023	-11.54	0.009	-55.00	0.006	-66.67	0.019	-13.64	0.099	-13.16

Table 10. Nitrate Stability over time at Specific Ranges	Frozen Preservation			Refrigerated Preservation		
Range of Nitrate Concentration	4 Days	7 Days	28 Days	4 Days	7 Days	28 Days
0.010mg/L - 0.024mg/L	No	No	No	No	No	No
1.438mg/L - 1.473mg/L	Yes	Yes	Yes	Yes	Yes	Yes
23mg/L - 26mg/L	Yes	Yes	Yes	Yes	Yes	Yes
30mg/L - 33mg/L	Yes	Yes	Yes	Yes	Yes	Yes

Table 11. Nitrate

		UTR		WFCR		SJR		SRF		FC		OC	
	Time	Nitrate (mg/L)	Percent Recovery (%)	Nitrate (mg/L)	Percent Recovery (%)	Nitrate (mg/L)	Percent Recovery (%)	Nitrate (mg/L)	Percent Recovery (%)	Nitrate (mg/L)	Percent Recovery (%)	Nitrate (mg/L)	Percent Recovery (%)
Field Filtered and Frozen	<48 hours	0.019		0.016		1.438		0.012		25.639		32.311	
	4 days	0.021	10.53	0.018	12.50	1.452	0.97	0.018	50.00	25.564	-0.29	32.401	0.28
	7 days	0.023	21.05	0.024	50.00	1.465	1.88	0.023	91.67	24.816	-3.21	32.207	-0.32
	28 days	0.021	10.53	0.020	25.00	1.468	2.09	0.021	75.00	23.980	-6.47	30.396	-5.93
Field Filtered and Refrigerated	<48 hours	0.014		0.015		1.459		0.010		24.907		32.227	
	4 days	0.020	42.86	0.020	33.33	1.452	-0.48	0.016	60.00	25.303	1.59	31.885	-1.06
	7 days	0.023	64.29	0.020	33.33	1.468	0.62	0.023	130.00	25.091	0.74	32.385	0.49
	28 days	0.022	57.14	0.019	26.67	1.473	0.96	0.021	110.00	24.396	-2.05	30.601	-5.05

Table 12. Total Nitrogen Stability over time at Specific Ranges	Refrigerated Preservation		
Range of Total Nitrogen Concentration	4 Days	7 Days	28 Days
0.146mg/L - 0.204mg/L	Yes	Yes	No
2.0mg/L - 2.5mg/L	Yes	Yes	Yes
24mg/L - 27mg/L	Yes	Yes	Yes
32mg/L - 35mg/L	Yes	Yes	Yes

Table 13. Total Nitrogen

		UTR		WFCR		SJR		SRF		FC		OC	
	Time	Total Nitrogen (mg/L)	Percent Recovery (%)	Total Nitrogen (mg/L)	Percent Recovery (%)	Total Nitrogen (mg/L)	Percent Recovery (%)	Total Nitrogen (mg/L)	Percent Recovery (%)	Total Nitrogen (mg/L)	Percent Recovery (%)	Total Nitrogen (mg/L)	Percent Recovery (%)
Refrigerated	<48 hours	0.163		0.164		2.154		0.176		24.500		32.800	
	4 days	0.151	-7.36	0.146	-10.98	2.146	-0.37	0.164	-6.82	25.314	3.32	34.414	4.92
	7 days	0.178	9.20	0.152	-7.32	2.079	-3.48	0.185	5.11	25.357	3.50	34.286	4.53
	28 days	0.204	25.15	0.155	-5.49	2.107	-2.18	0.169	-3.98	26.857	9.62	34.840	6.22

Table 14. Total Phosphorus Stability over time at Specific Ranges	Refrigerated Preservation		
Range of Total Phosphorus Concentration	4 Days	7 Days	28 Days
0.012mg/L - 0.050mg/L	No	No	No
0.156mg/L - 0.182mg/L	Yes	No	No
0.822mg/L - 2.561mg/L	No	No	No

Table 15. Total Phosphorus

		UTR		WFCR		SJR		SRF		FC		OC	
	Time	Total Phosphorus (mg/L)	Percent Recovery (%)	Total Phosphorus (mg/L)	Percent Recovery (%)	Total Phosphorus (mg/L)	Percent Recovery (%)	Total Phosphorus (mg/L)	Percent Recovery (%)	Total Phosphorus (mg/L)	Percent Recovery (%)	Total Phosphorus (mg/L)	Percent Recovery (%)
Refrigerated	<48 hours	0.036		0.031		0.178		0.053		0.035		0.822	
	4 days	0.040	11.11	0.031	0.00	0.182	2.25	0.048	-9.43	0.012	-65.71	1.668	102.92
	7 days	0.045	25.00	0.033	6.45	0.156	-12.36	0.046	-13.21	0.033	-5.71	2.013	144.89
	28 days	0.044	22.22	0.036	16.13	0.158	-11.24	0.039	-26.42	0.032	-8.57	2.561	211.56

Table 16. Holding Time Extension Summary

Analyte	Frozen Preservation	Refrigerated Preservation	Reporting Limit
Soluble Reactive Phosphorus	Holding Time can be extended up to 4 days.	Holding Time can be extended up to 4 days.	0.050 mg/L
Nitrite	Holding Time cannot be extended.	Holding Time cannot be extended.	0.020 mg/L
Nitrate+Nitrite	Holding Time can be extended up to 4 days.	Holding Time cannot be extended.	0.050 mg/L
Ammonia	Holding Time cannot be extended.	Holding Time cannot be extended.	0.020 mg/L
Nitrate	Holding Time cannot be extended.	Holding Time cannot be extended.	0.020 mg/L*
Total Nitrogen	Holding Time can be extended up to 28 days.	Holding Time can be extended up to 28 days.	0.050 mg/L
Total Phosphorus	Holding Time cannot be extended.	Holding Time cannot be extended.	0.050 mg/L

*assumed to be the same as Nitrite because it is a calculated value

Figure 1. Soluble Reactive Phosphorus Frozen

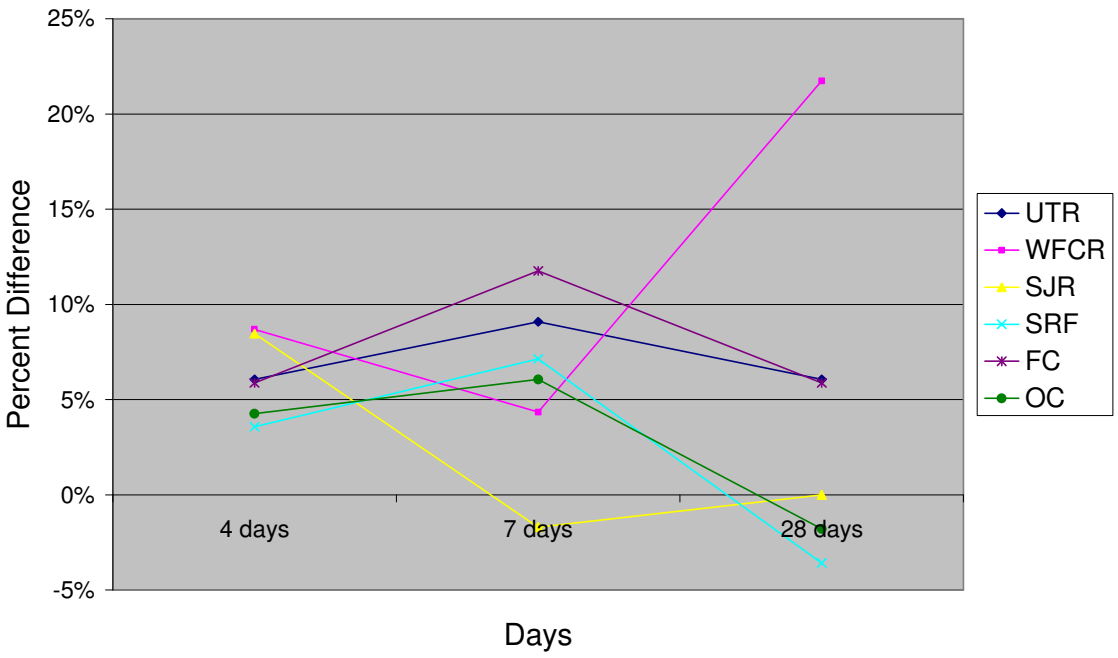

Figure 2. Soluble Reactive Phosphorus Refrigerated

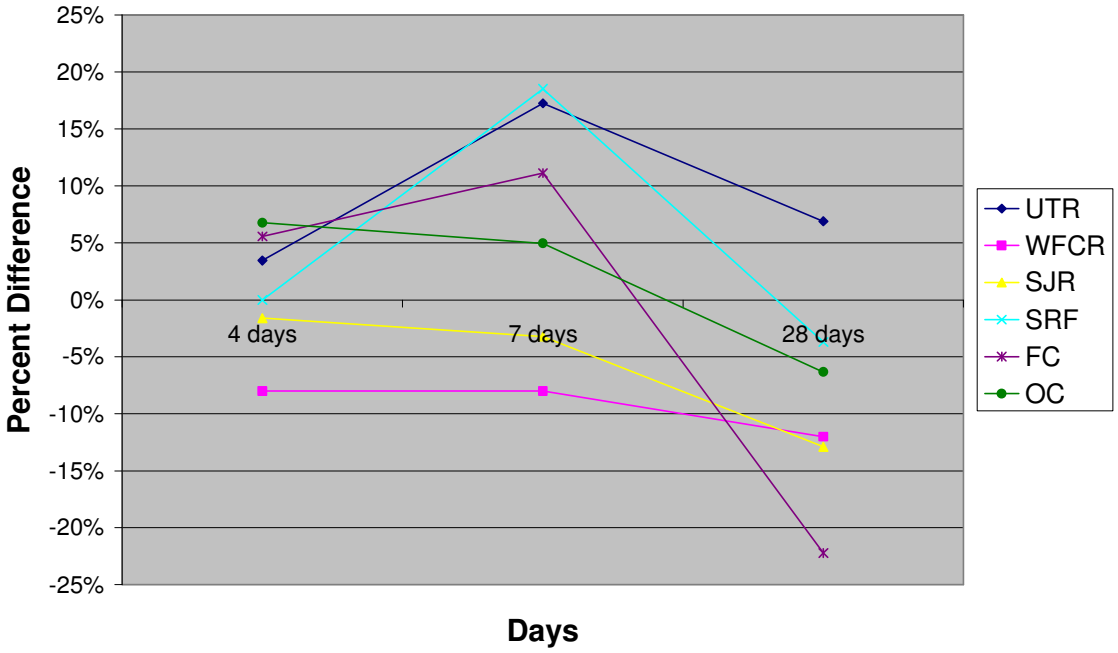

Figure 3. Nitrite Frozen

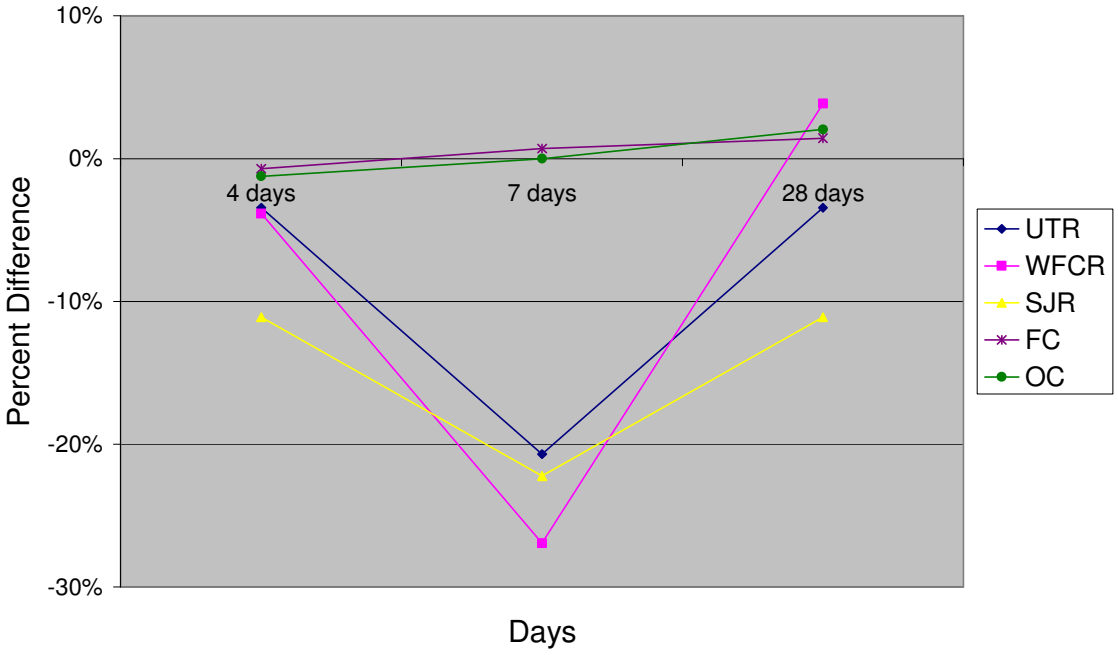

Figure 4. Nitrite Refrigerated

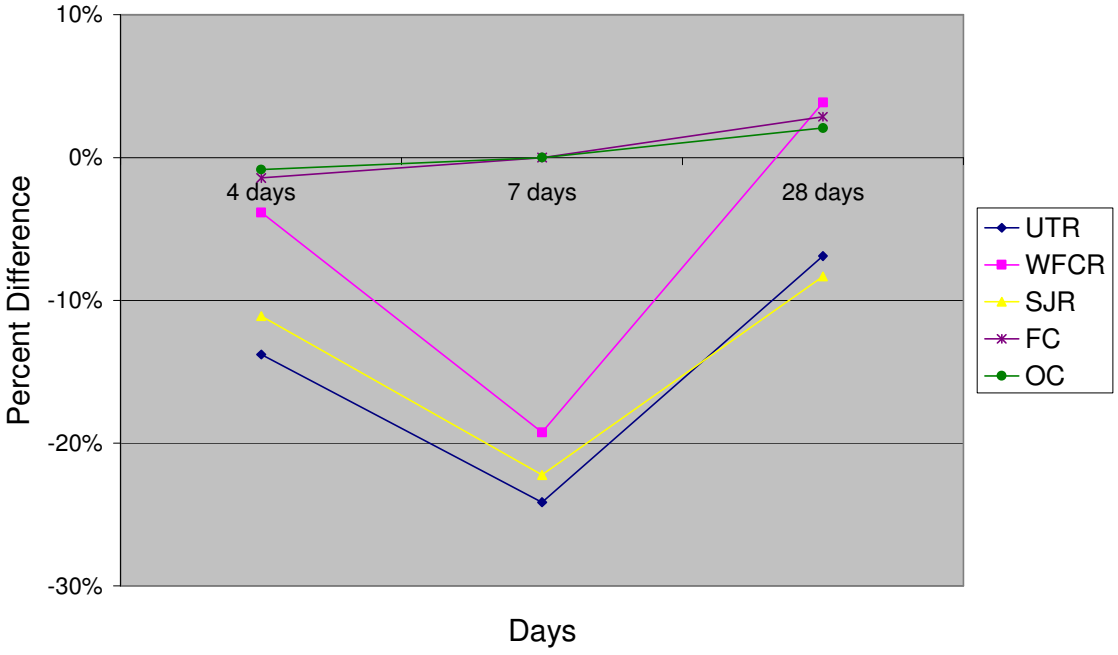

Figure 5. Nitrate+Nitrite Frozen Preservation

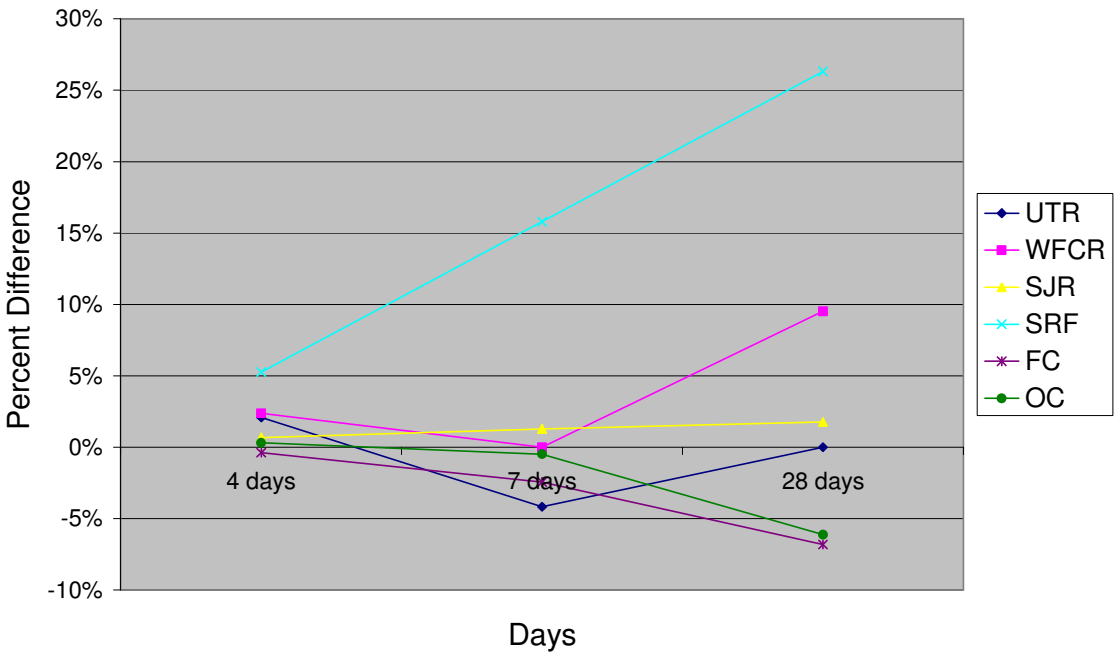

Figure 6. Nitrate+Nitrite Refrigeration Preservation

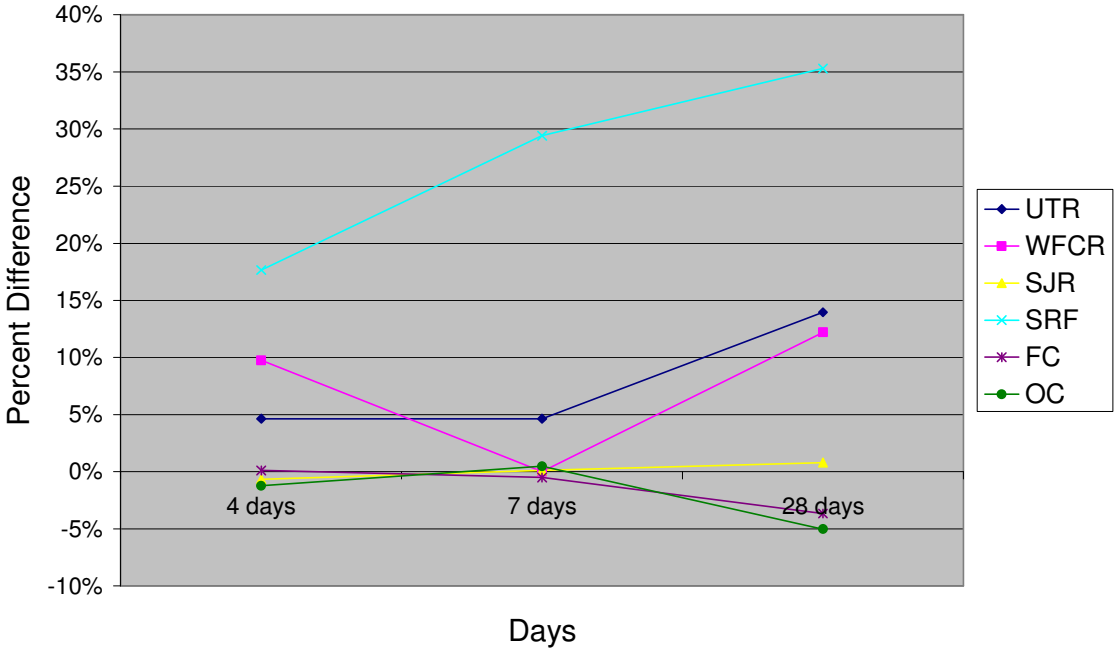

Figure 7. Ammonia Frozen Preservation

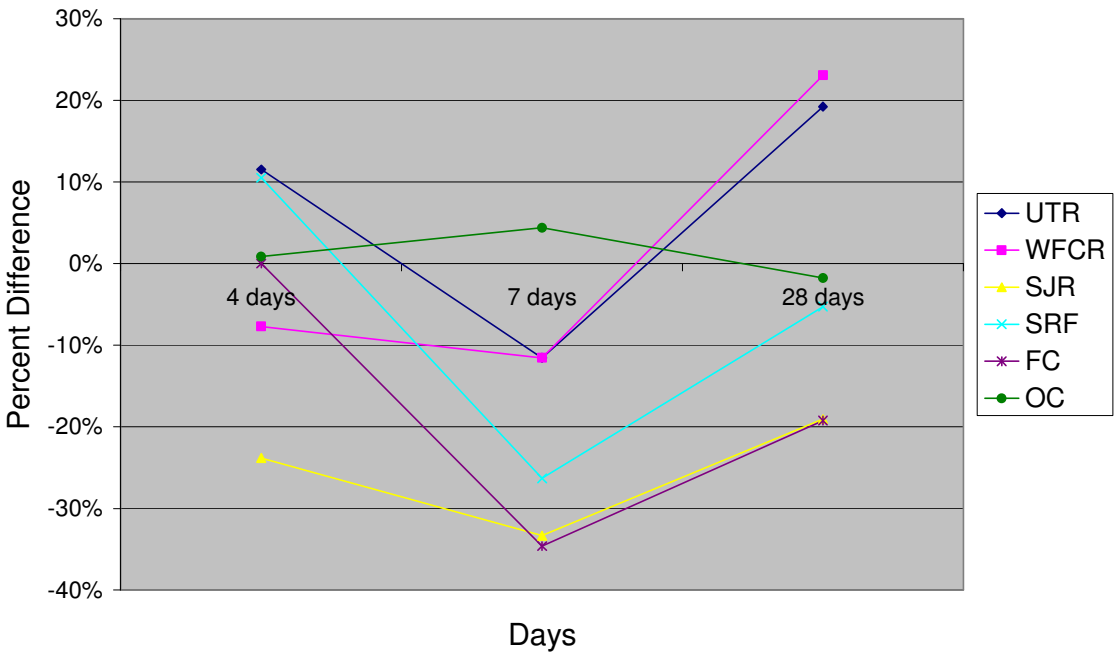

Figure 8. Ammonia Refrigeration Preservation

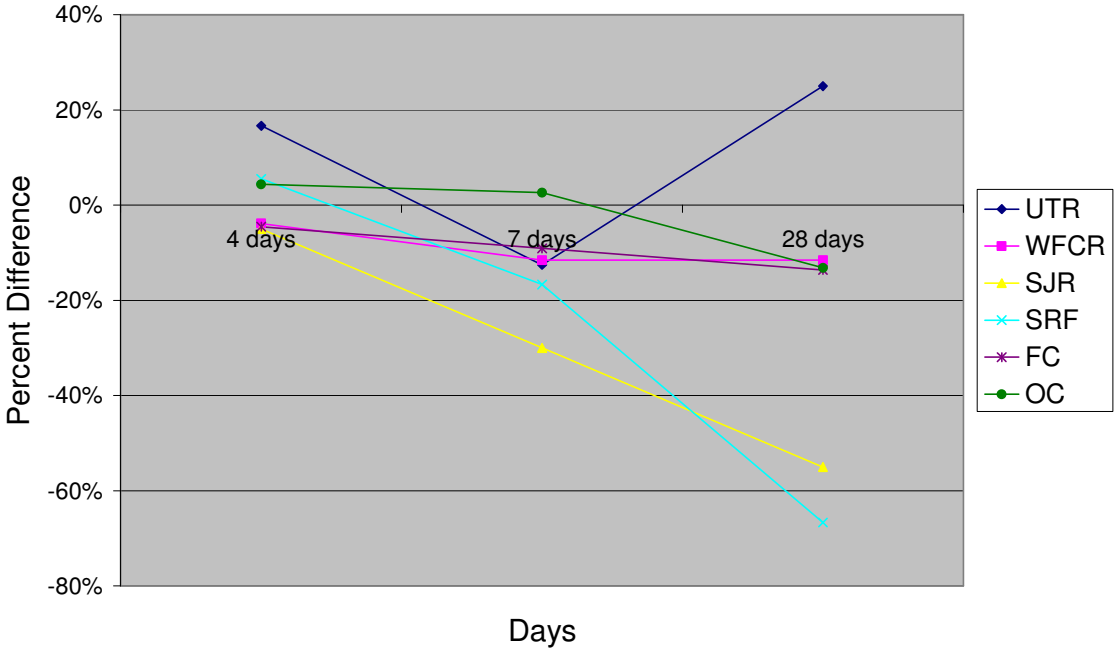

Figure 9. Nitrate Frozen Preservation

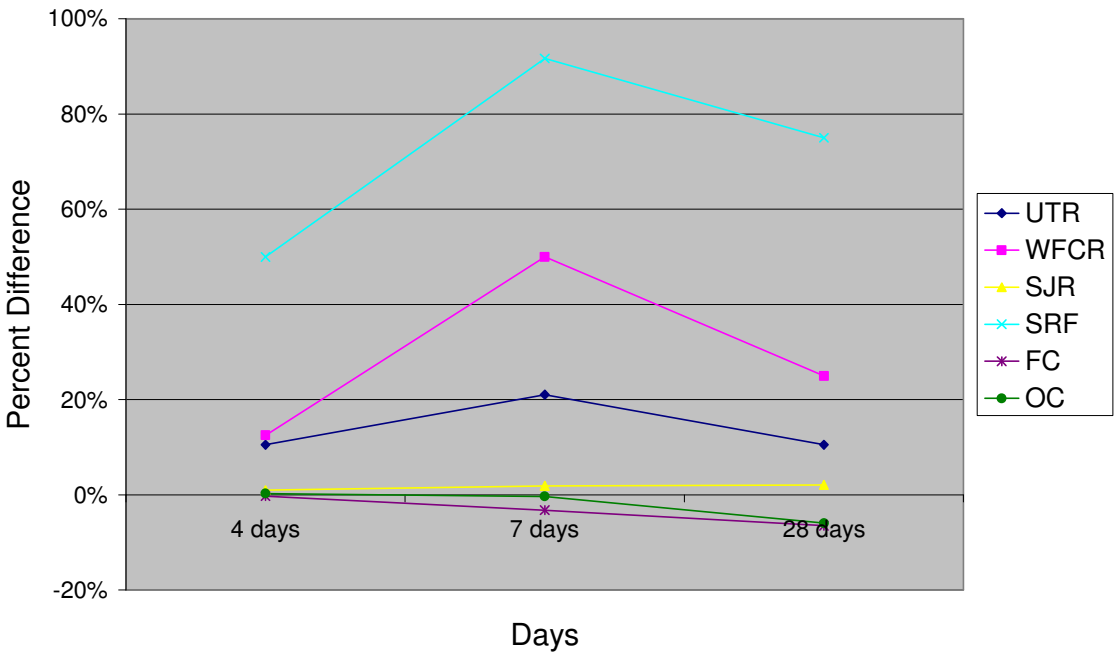

Figure 10. Nitrate Refrigeration Preservation

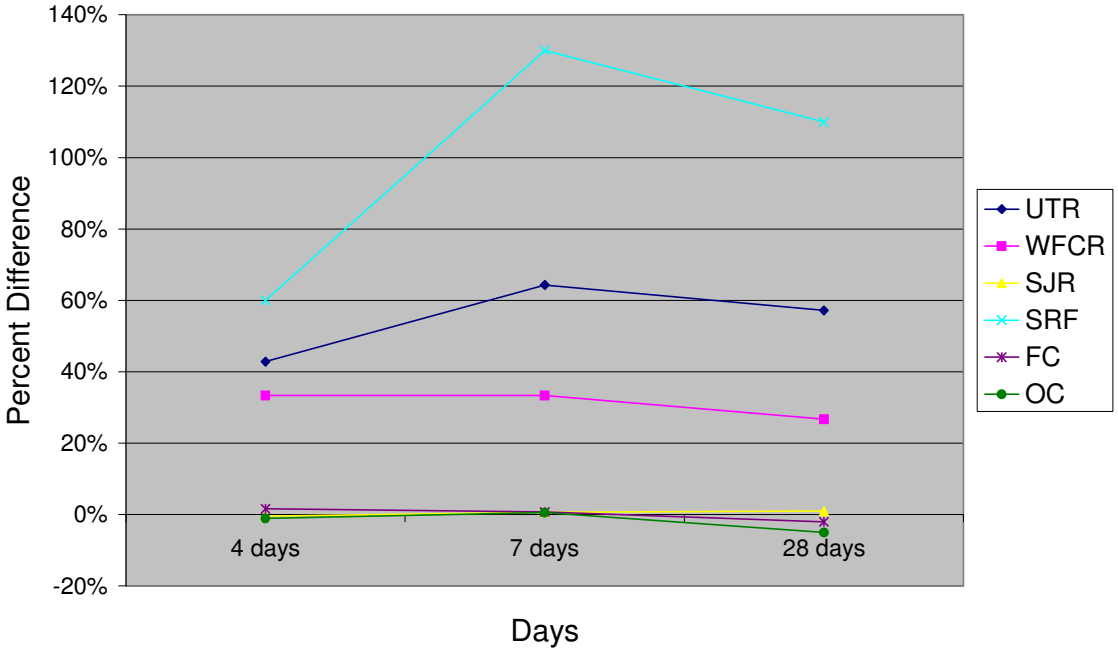

Figure 11. Total Nitrogen

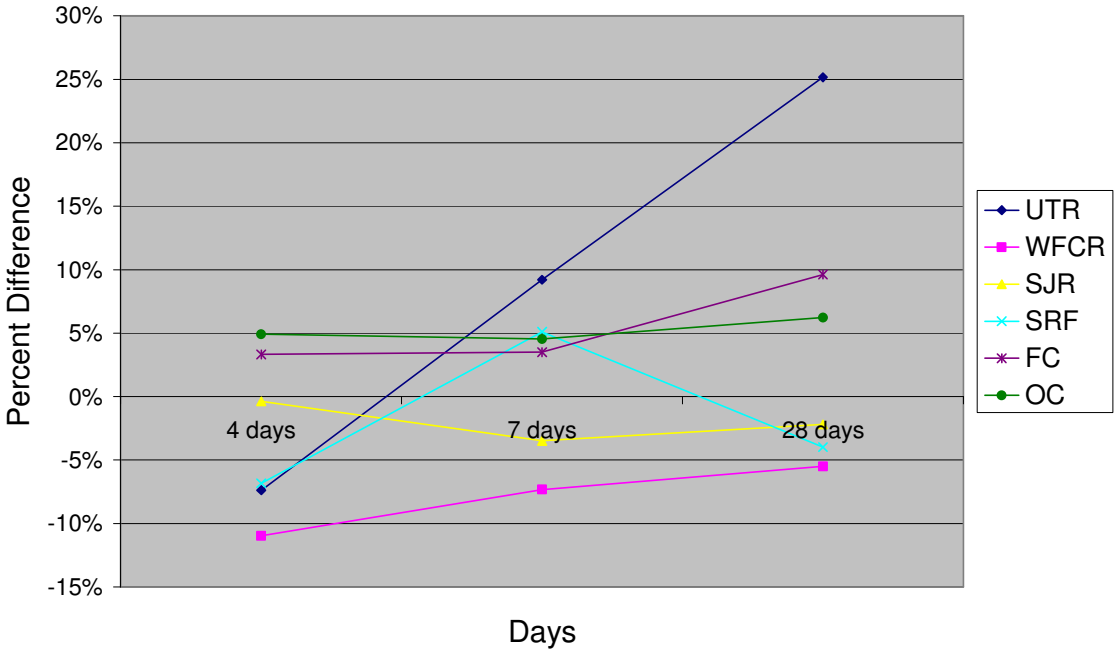

Figure 12. Total Phosphorus